SHORT COMMUNICATION

Seasonal variations of the humoral immune parameters of European sea bass (*Dicentrarchus labrax* L.)

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Abstract

Seasonal cycles, mainly due to great variations in the light duration and temperature, are important and modulate several aspects of the animal behavior. In the case of poikilotherms animals such as fish this is very relevant. Thus, temperature changes fish immunity and affects disease resistance. We evaluate in this work the season variations of the European sea bass (*Dicentrarchus labrax*) humoral innate parameters focusing on winter months, at which the culture of this specie is more difficult. Our results showed that not all the innate immune parameters are depressed by low temperatures. Moreover,

some of them are more dependent than others to the season and both temperature and photoperiod are operating together.

Seasonality dominates the life cycle of animals, mainly in the case of poikilotherms such as fish. It is now widely demonstrated that season cycles affect to fish physiology including animal behavior, body weight and food intake, reproduction, locomotor activity or immunity [1]. Several natural factors may influence these cycles but photoperiod and temperature are the most important. Focusing on temperature, it is generally accepted that the innate immune response is independent of the temperature whilst the adaptive response is dependent on it. However, low temperature has been associated with an increase in fish susceptibility to disease [2, 3] suggesting that both innate and adaptive immunity are affected by temperature. Thus, the influence of temperature on fish immunity has been described and varied with the fish species [4-6]. Otherwise, temperature also represents a crucial factor in the survival and infective ability of several pathogens, as occurs with viral hemorrhagic septicemia virus (VHSV), some *alphavirus* or some bacteria such as *Vibrio sp*, *Flavobacterium psychorophilum* or *Moritella viscose* which cause disease when water temperature drops [7-11]. All this data highlight the relevance of water temperature in fish health, but little is known in the case of Mediterranean aquaculture species, mainly gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). In the case of gilthead seabream, it was reported that the complement and agglutination activities of serum were lowest in winter months whilst the lysozyme activity was similar during all the year [5]. In sea bass, there is only a study reporting the effect of seasonality on cortisol, hematocrit, leucocrit, serum lysozyme activity and total glutathione [12]. Due to the economical importance of the European sea bass in the Mediterranean aquaculture and the season cycles on fish immunity and health, we propose in this study the evaluation of several humoral innate parameters of European sea bass at different seasons but focusing on winter, the most challenging for fish culture.

Healthy specimens of European sea bass were bred at the *Centro Oceanográfico de Murcia* with natural conditions of photoperiod, temperature, salinity and aeration. One year old fish were sampled once a month (n=10 fish/month) from September (121±13 g body weight (bw)) to May (225±21 g bw). The specimens were bred with a commercial pellet dry diet (Skretting) *ad libitum*. Temperature, mortality and food

intake were recorded daily. Mean temperature in each month was represented. The specimens were anesthetized with 40 µl L^{-1} of clove oil before handling. The blood was obtained from the caudal peduncle and the serum samples, obtained by centrifugation (10,000 g, 1 min, 4 ºC), immediately frozen in liquid nitrogen and stored at -80 ºC until use. Protease activity was determined as the percentage of hydrolysis of azocasein by 2 mg/ml of proteinase K [13]. Total anti-protease activity was determined as the percentage of inhibition of the hydrolysis of azocasein by 2 mg/ml of proteinase K [14]. Hemolytic activity of complement was assayed using sheep red blood cells (SRBC; Biomedics) as targets [15]. The results were expressed in ACH_{50} units, as the titre at which 50 % haemolysis is produced [16]. Lysozyme activity was measured according to a turbidimetric method that uses the lysis of *Micrococcus lysodeikicus* for determination of the lysozyme activity using hen egg-white lysozyme as standard [17]. Serum antibacterial activity was determined by evaluating the inhibition on the bacterial growth of *Vibrio harveyi* curves [16]. The peroxidase activity was measured with a method previously described [18]. Data were presented as mean ± S.E.M (n = 10) and significance analysed by ANOVA ($P<0.1$) and Waller-Duncan post-hoc test.

Our results showed that most of the activities of the humoral innate response had a temperature independent pattern or no statistically significant changes. Though protease activity showed the lowest level in December, however, the variations are very little in value and probably not biologically significant (Fig. 1a). The antiprotease activity showed a decrease from December to April when temperature range from 15ºC to 20ºC. Similarly, the hemolytic activity of complement showed seasonal variation, but not subjected to temperature changes as lowest activity was marked during early autumn (September and October) when temperature was around 20 ºC as also occurred in early spring, when the highest levels of hemolytic activity were recorded (Fig. 1c). This pattern of variation is similar to the pattern observed in Asian catfish (*Clarias batrachus*), but contrary to the one observed in gilthead seabream (*Sparus aurata*), where a seasonal pattern related to temperature was found with the lowest values recorded in winter, however, big differences were also observed between the different sampling points during this period [5, 6]. Bactericidal and peroxidase activities were not clearly related with temperature. Thus, bactericidal activity reached the highest levels at different time point throughout the time analyzed (Fig. 1d). Similar situation was found in the peroxidase activity, which the minimum level occurred in early autumn (October)

and the maximum levels were recorded in December, but also in April when the water temperatures were very similar to the temperature recorded in October (Fig. 1e). These variations not related with the temperature could be explained due to daylight changes which have been demonstrated to affect leukocyte distribution and numbers, serum lysozyme activity or specific antibody levels in several fish species (see review [4]). Furthermore, in a day-night cycle, sea bass showed variations in the complement activity but not in the peroxidase or lysozyme activities [19]. However further studies will be needed to clearly determine the role of photoperiod in the innate immune responses of European sea bass. Otherwise, lysozyme activity showed no changes through the sampling time (Fig. 1f). Interestingly, previous studies showed that serum lysozyme activity in the European sea bass decreases when temperatures drop to 5 ºC [12], however our data showed no changes of lysozyme activity as the lowest temperatures were around 15 ºC. All these data together suggest that lysozyme activity in European sea bass have a temperature dependent pattern whenever winter is more accused.

In conclusion, our data indicate that some innate immune parameters are more dependent than others to the season and both temperature and photoperiod are operating together. Moreover, it is worthy to note that not all the parameters are decreased in cold months indicating that the immune system is not seriously depressed at this season. This knowledge could be interesting for research and aquaculture purposes.

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Figure 1: European sea bass humoral immune parameters and temperature at the indicated months. Protease (a), antiprotease (b), haemolytic (c), bactericidal (d), peroxidase (e) and lysozyme (f) activities. Data represent means ± standard error (n = 10). Different letters denote statistically significant differences between the groups according to ANOVA and a Waller-Duncan post-hoc test (P≤0.1).

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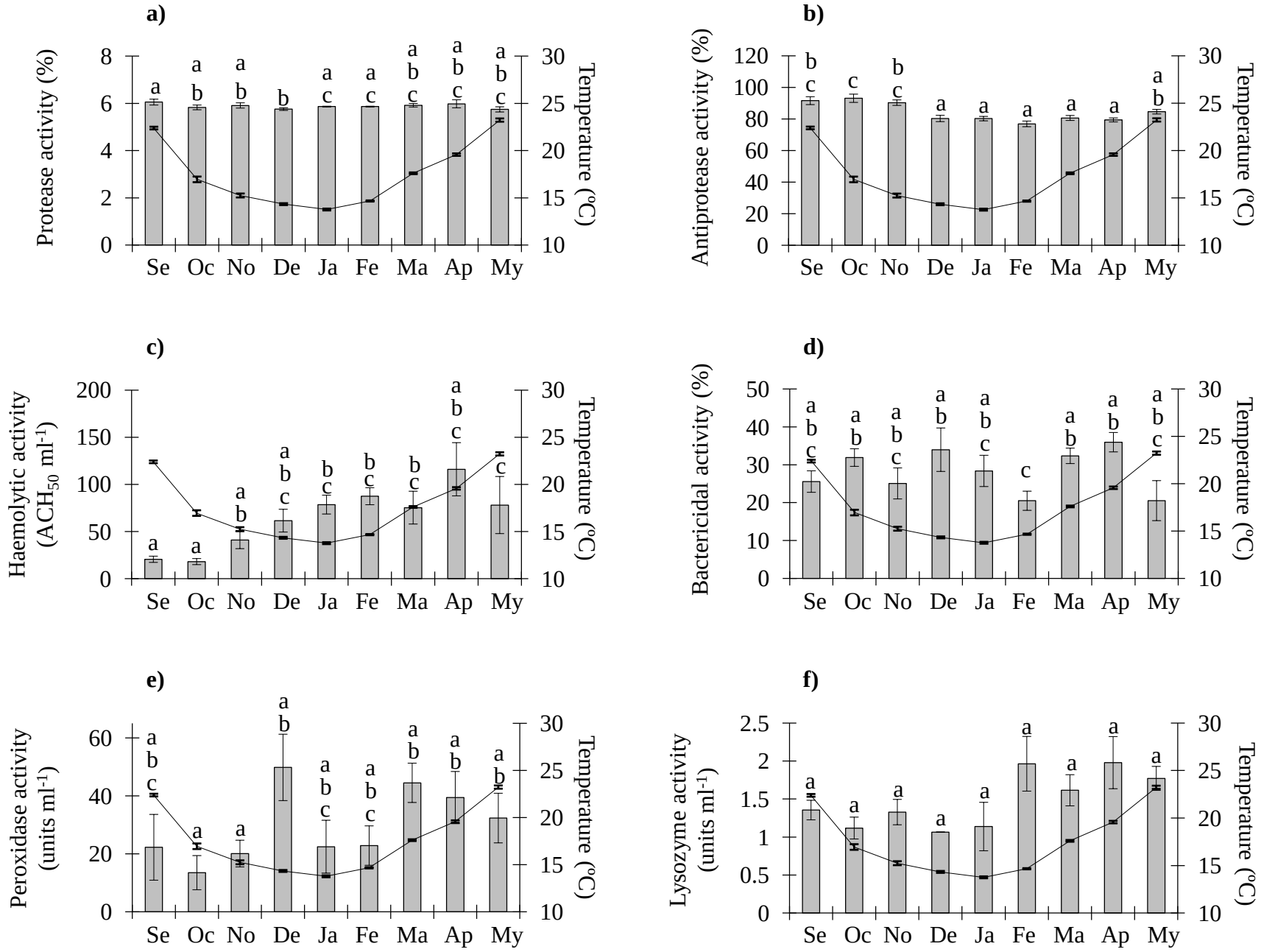
a)
Protease activity (%)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My
b)
Antiprotease activity (%)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My
c)
Haemolytic activity (ACH50 ml-1)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My
d)
Bactericidal activity (%)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My
e)
Peroxidase activity (units ml-1)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My
f)
Lysozyme activity (units ml-1)
Temperature (ºC)
Se Oc No De Ja Fe Ma Ap My